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NUTRITION REVIEWS

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NUTRITION REVIEWS

VOL. 2 OCTOBER, 1944 No. 10

FATS IN METABOLISM

The early metabolic story of the food fats of either animal or vegetable origin is relatively short and simple. Fats are, with few exceptions, well digested, well absorbed, and well utilized if taken in moderate amounts with other foods. When taken in larger proportions in the food, fats cause disturbances which are the result of several factors. They coat or saturate other food materials and in that way slow the penetration of the water soluble digestive enzymes. They slow the enzymatic and muscular activity of the stomach, retard its emptying, and may produce discomfort and finally regurgitation of intestinal contents into the stomach. Fat may cause diarrhea due probably to the volume of liquid that it produces when melted or to the fact that the fatty acids or soaps produced by digestion are irritating to the intestine. The reason for the similar and simple behavior of the various fats as noted above rests in the fact that they are composed to the extent of 70 or 80 per per cent of the same few fatty acids of which oleic and palmitic are quantitatively the most important.

One exception to the general rule of simplicity of composition to which special attention is being given is that of cow milk butter. Due to the shortage and rationing, together with certain efforts to hamper the sale of substitutes, this fat has considerable popular as well as scientific interest. Butter has a large content of unusual fatty acids, especially those of greater or less than ordinary (C_{14} to C_{18}) chain length. Special value has been claimed (Boutwell, Geyer, Elvehjem, and Hart, *J. Dairy Sci.* **24,** *1027 (1941)*) and denied (Deuel, Movitt, Hallman, and Mattson, *J. Nutrition* **27,** *107 (1944)*) for cow milk fat as compared with other fats as a food for growing animals, and the verdict at present rests in doubt. The presence in butter of relatively short chain unsaturated fatty acids (C_{10} to C_{14}) with the double bond in the ordinary C_9 to C_{10} position (Bosworth and Brown, *J. Biol. Chem.* **103,** *115 (1933)*; Hilditch and Paul, *Biochem. J.* **30,** *1905 (1936)*) has considerable theoretical interest since it may be regarded as evidence of oxidation from the methyl end of the fatty acid chain instead of from the carboxyl end as has ordinarily been assumed. Continued oxidation from the methyl end past the double bond would result in short chain saturated acids (C_4 to C_8) which are also characteristic of butter. Oxidation from the methyl end in glycerides was believed (Flaschenträger and Bernhard, *Z. physiol. Chem.* **238,** *221 (1936)*) to account for the dicarboxylic acids found in urine after feeding the fatty acids of intermediate chain length (Verkade and Van Der Lee, *Biochem. J.* **28,** *31 (1934)*).

The efforts to prevent the competition of margarine with butter on the assumption that there is something especially valuable and divine in butter that is not present in any "man-made product" do not especially impress the biochemist. Fats of milk, except for their vitamin A content, have not been proved superior to other less expensive fats, and vitamin A can be found in less expensive foods than milk (Wilder and Keys, "Handbook of Nutrition," *Am. Med. Assn. p. 279, Chicago (1943)*). The delicious taste of butter assures its marketing and use to the extent of its availability without other aid.

While the early metabolic story of the food fats is relatively short and easy, their later history, the part they and their compounds later take in the functions of the living body is complicated and little understood. Fatty acid compounds, largely phospholipids, make

up nearly half the dry substance of the brain, and are large constituents of other important organs such as liver, lungs, adrenals, and kidney, and smaller but still important components of the heart and to a less extent of other muscles (Kaucher, Galbraith, Button, and Williams, *Arch. Biochem.* **3,** *203 (1943)*). The considerable amount of work which has been done on these lipids has little more than outlined the field of their occurrence and function. New members of the group are appearing, e.g., serine and inositol cephalin (Folch and Woolley, *J. Biol. Chem.* **142,** *963 (1942)*) and the group of aldehyde phospholipids (in which a single fatty aldehyde takes the place of two fatty acid molecules on the glycerol of the phospholipids (Neuman, *Abstracts, Div. Biol. Chem., Am. Chem. Soc. 106th Meeting, Pittsburgh,* **30B** *(1943)*; Anchel and Waelsch, *J. Biol. Chem.* **145,** *605 (1942)*). The importance of this latter group of phospholipids is indicated by the fact that it makes up about 10 per cent of the phospholipids of brain and muscle, while it is practically absent from liver. It seems to be an intermediate product and is so unstable that a good deal of it is lost during extraction unless the tissue has been dried frozen immediately after removal from the animal. The possibilities of sphingomyelin as a tissue constituent and as a possible transport mechanism for fatty acids have been developed by Thannhauser and Reichel (*J. Biol. Chem.* **135,** *1 (1940)*). Among the other complex lipids, cholesterol esters have received attention (Kelsey and Longenecker, *Ibid.* **139,** *727 (1941)*; Sperry and Stoyanoff, *Ibid.* **126,** *77 (1938)*). These have special significance because of the fact that the fatty acids in them are more unsaturated than those of either the fat or phospholipid.

The importance of the more complex lipids, especially the phospholipids, rests largely in the fatty acids combined in them, although some of their other constituents, for example choline, especially in its relation to fatty livers and hemorrhagic degeneration of the kidneys, has a considerable place in the current literature (McHenry and Patterson, *Physiol. Rev.* **24,** *128 (1944)*). One phase of choline metabolism, its relation to the methyl group, brings it into competition with other methyl-containing compounds, as for example methionine and creatin, and this has contributed much interesting investigation (du Vigneaud, Chandler, Cohn, and Brown, *J. Biol. Chem.* **134,** *787 (1940)*; Dolby, Nunn, and Smedley-MacLean, *Biochem. J.* **34,** *1422 (1940)*). The fatty acids in the phospholipids include the essential fatty acids, especially arachidonic and with it those acids, mainly linoleic, from which arachidonic is made. Incidentally, these "essential" fatty acids have been conclusively shown to be essential for only one species, the rat, although certain skin symptoms in human beings have been cured by feeding these acids (Hansen, *Am. J. Dis. Child.* **53,** *933 (1937)*), and it has been demonstrated in these cases that the amount of these acids in the blood is below normal. Whether the lack of these fatty acids is ever serious in human beings is a question, since they appear to be universally present in natural foods, even starch containing a considerable amount.

Vegetable fats can in general be supplied at a lower cost than animal fats, and the chemist has learned the trick of modifying them to produce fats of almost any desired properties. The economics of feeding grains to animals to produce animal fat and animal protein has been criticized, since the animal is not an efficient converter of protein and carbohydrate into meat and fat. Moreover, the food fat, whether animal or vegetable, undergoes important changes during digestion and absorption. There may be a rearrangement of fatty acids in the glyceride molecule to approach the natural ideal that in every glyceride molecule there shall be represented the greatest possible number of different fatty acids (Hilditch's rule). The

mechanism and physiologic significance of this phenomenon offers an interesting and fruitful field for investigation. Other changes which may be brought about during absorption and preparatory metabolism of the fatty acids are a limited lengthening or shortening of the chain and the introduction of a double bond into a saturated chain.

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AVIDIN, BIOTIN, AND LYSOZYME

An important experimental finding appears to have resulted from the suggestions of a man who is primarily a scientific writer rather than an experimentalist. William Laurence, as the result of careful examination of the literature, was struck by the similarity in properties and distribution of two presumptively unrelated substances, avidin and lysozyme. Avidin which occurs in abundance in egg white has the property of combining stoichiometrically with biotin and rendering it unavailable for either experimental animals or microorganisms. Lysozyme has the property of lysing certain microorganisms, e.g., Micrococcus lysodeikticus and Sarcina lutea. Both avidin and lysozyme are basic proteins and occur in the same fraction of egg white. Laurence instigated work to test a relationship between these two substances. Preliminary results of this work have now been published (Meyer, *Science* **99,** *391* (*1944*) and Laurence, *Science* **99,** *392* (*1944*)).

Laurence made a number of comparisons between the lysozyme and avidin activities of various "lysozyme" and "avidin" preparations. Not only was lysozyme activity present in concentrated avidin preparations, and vice versa, but both types of activity were essentially parallel in diverse preparations.

Karl Meyer extended these studies by observing the effect of biotin on the lysozyme activity of several different preparations. Although some lysozyme activity was always present in the original preparation, the addition of biotin increased the activity manifold. In one instance the original activity was 640 units per milligram. The addition of 1 microgram of biotin increased the activity to 5000 units per milligram, and 10 micrograms of biotin brought the activity to 164,000 units per milligram, a two hundred fiftyfold increase. This strongly suggests that avidin plus biotin equals lysozyme. And, although there are some difficulties in accepting this simple conclusion, a close relationship between avidin, biotin, and lysozyme must certainly exist. It is perhaps misleading to continue to designate avidin as "antibiotin" since it "more likely serves as a biotin-carrier and thus may be more properly termed a 'probiotin,' its so-called 'toxic' effect being due to other reasons, such as molecular size, resulting in its non-absorption from the gastro-intestinal tract." (Laurence). György and Rose (*Science* **94,** *261* (*1941*)) observed that although avidin (in part combined with biotin) was detrimental to biotin deficient animals when fed orally, it exerted a beneficial effect when given parenterally.

There are some points which further work will undoubtedly clarify. The amount of biotin required to give a maximum lysozyme activity far exceeds the stoichiometric amount required to combine with all of the avidin present. That this cannot be simply a mass action effect on a dissociable avidin-biotin complex is indicated by the failure of

dialysis to inactivate lysozyme as would be expected for an easily dissociable combination. It is conceivable that the prosthetic group in lysozyme is a biotin derivative rather than biotin itself, and that free biotin protects this prosthetic group. There is precedent for this possibility both in the case of nicotinamide and of thiamin. There remains the possibility that biotin, instead of activating the enzyme, in some manner renders the test microorganism more susceptible to the action of lysozyme.

It will be highly interesting to follow further progress of these studies, to know whether the other biotins which are known to exist are active in stimulating lysozyme activity. Lysozyme belongs to an important family of mucolytic enzymes. Indeed, lysozyme-susceptible organisms possess similar enzymes which are thought to be involved in cell division. An active development of this field may be expected to result from the important findings of Meyer and Laurence and will be awaited with great interest.

RELATION OF METHIONINE AND PROTEIN DEFICIENCY TO MAPHARSEN LIVER INJURY

Although the earliest studies of the relation of nutritional factors to protection against liver injury by chemicals stressed the importance of carbohydrates, more recent investigations are placing more and more emphasis on the significance of protein. Thus Schifrin (*Virchows Arch. f. path. Anat.* **287,** *175 (1932)*) called attention to the value of protein in protecting the liver against arsphenamine injury, and Goldschmidt, Vars, and Ravdin (*J. Clin. Invest.* **18,** *277 (1939)*) indicated the importance of a high protein diet in protecting rats against damage by chloroform. The report by Miller, Ross, and Whipple (*Am. J. Med. Sci.* **200,** *739 (1940)*) laid emphasis on the sulfur-containing amino acids, methionine and cystine, as the specific protein factors giving protection, the former being more effective than the latter. The significance of protein and of the sulfur-containing amino acids in preventing the development of cirrhosis of the liver has been discussed in previous reviews (*Nutrition Reviews* **1,** *88 (1943)*; **2,** *180 (1944)*).

Goodell, Hanson, and Hawkins (*J. Exp. Med.* **79,** *625 (1944)*) report studies in dogs which will be of interest to clinicians concerned with the treatment of syphilis. Active healthy adult dogs were fasted for one week and were then placed on a diet consisting of carbohydrate 72.5 per cent and fat 20.2 per cent. Vitamins and minerals composed a large part of the remainder and thus the quantity of protein in the diet was low. In a few of the animals plasmapheresis was employed to effect a more rapid protein depletion. Dogs depleted of protein by diet alone were maintained on the dietary regimen for five to ten weeks while in those instances in which plasmapheresis was employed, a low level of plasma protein (usually under 5 g. per cent) was reached in two to six weeks.

The object of the experiment was to determine the susceptibility of protein depleted animals to liver injury produced by injections of mapharsen and to observe any possible protective effect by methionine. Mapharsen was chosen because it is the arsenical now most commonly used in the treatment of syphilis. In man the dose used is approximately 0.001 g. per kilo. The dogs were given amounts varying from 0.0025 to 0.0045 g. per kilo. Since previous experiments had indicated to the investigators that histologic examination of the liver is not as good an index of the degree of hepatic damage as measurements of physiologic function, more reliance was placed on the icterus index than on anatomical findings in

judging the presence and degree of liver injury.

The dogs were divided into three main groups. The first group was given no methionine. The second group received this amino acid while in the third group the effects of mapharsen were observed alternately without and after administration of methionine.

In a control experiment on one dog maintained on the regular kennel ration of kitchen scraps, the plasma protein of which varied from 5.79 to 6.89 g. per cent, the injection of doses of mapharsen as high as 0.0045 g. per kilo caused no jaundice or obvious toxic reactions. Following a dose of 0.007 g. per kilo the dog died, but the only noteworthy anatomical finding was congestion of the viscera.

In 5 dogs given no methionine, a dose of mapharsen as low as 0.002 g. per kilo produced definite icterus. Larger doses caused more marked icterus. The icterus index in one animal, for example, was as high as 19 following the injection of 0.0025 g. per kilo. In this animal fatty degeneration of the liver was observed.

On the other hand, in 3 dogs given 3 to 6 g. of methionine in the regular feeding the day prior to the administration of mapharsen, no icterus was observed when doses as high as 0.0045 g. were given. In the third group of 3 animals doses of mapharsen of 0.0025 g. were found to produce marked icterus whereas after 2 to 4 g. of methionine were fed the day before, doses almost twice as great (0.0045 g.) produced no icterus and no increase of the traces persisting after previous injections in the unprotected state.

The authors found that methionine given intravenously in doses of 1 g. immediately prior to the mapharsen was inadequate for uniform protection. They did not determine whether doses as high as those fed by mouth would give protection if given intravenously immediately before the arsenical.

Although the number of animals used is small, in the light of previous reports these data are rather convincing. The importance of methionine in clinical medicine has yet to be demonstrated, but from these studies it would seem to be promising. These studies are of particular importance in view of the developing practice in antisyphilitic arsenical therapy of giving "massive" doses and intensive treatment. If the role of methionine is the same in man as in the dog, and if protein deficiency increases the susceptibility to liver injury by toxic agents, we have an explanation for the varying susceptibility of different individuals to the potentially toxic effects of arsenicals, chloroform, and other substances injurious to the liver. There arises also the possibility of treatment by giving methionine, as has already been described in one case by Beattie, Herbert, Wechtel, and Steele (*Brit. Med. J.* **1,** *209 (1944)*). In this instance an aviator who accidentally consumed a large amount of carbon tetrachloride, and developed severe liver damage, made a surprisingly rapid recovery following treatment with methionine. Judging by the quantity of carbon tetrachloride consumed, one would have expected death to have occurred.

DIET AND DISEASE IN THE BANTU

It has long been known that the incidence of disease among the Bantu (South African Negro) is different from that described for the whites or Europeans in South Africa. The following information has been taken from an article by Gilbert and Gillman (*Science* **99,** *398 (1944)*). "Cirrhosis of the liver is found in four out of five autopsies performed on male Bantu at the Johannesburg General Hospital." Primary carci-

noma of the liver, a rare disease in Europeans, accounts for 90.5 per cent of all cases of cancer among the Bantu working on the Witwatersrand Gold Mines. In the urban Bantu primary carcinoma of the liver occurs almost exclusively among young male adults in whom it accounts for 31.9 per cent of all carcinomas of the organs. Gallstones and diabetes are rare diseases. Peptic ulcer is seven times more prevalent among the whites than among the Bantu. Tuberculosis is widespread among the Bantu with a mortality rate of 50 per cent of admitted cases.

Such difference in incidence of disease has usually been attributed to racial characteristics, lack of sanitation, or natural resistance. Undoubtedly these factors do play a part in the condition. However, Gilbert and Gillman have considered the state of nutrition as a possible factor and present preliminary experimental evidence to support this point.

The Bantu is poor and lives largely on corn. In some areas this is supplemented by fermented milk. Meat and other foods are rarely eaten; as a consequence, deficiency diseases are widespread.

The effect of such a diet on animals has been tested. One hundred and twenty-five albino rats were kept on corn pap and fermented milk for a period of two years. As was to be expected, growth was much slower in the test animals as compared with the controls receiving a mixed diet. The latter doubled their weight in three weeks, whereas the corn fed animals had not reached this weight in seven weeks. However, no other significant differences were noted after five months. After fifteen months the test animals had lost much of their hair, their corneas were scarred and filled with capillaries, and numerous dental defects such as irregular incisor growth had occurred. Although a few litters were born, they were never reared.

On autopsy, Gilbert and Gillman report that liver lesions were present without exception. This varied from marked fatty changes to multilobular cirrhosis. Frank cirrhosis occurred in 20 per cent of the rats and affected almost exclusively the left lobes. The testes of all male rats were extensively damaged. In many animals hypertrophy and hemorrhage of the adrenals were present. Atrophy of the thyroid was common while the parathyroids were invariably enlarged. The parotid was always damaged while the pancreas was affected in 56 per cent of cases. Enlargement of the heart was found in those rats with extensive liver damage. Cirrhosis was less common in females. Although the skull was thick the bone cut easily. Other than the cirrhosis, the pathologic picture is not that clearly due to any specific deficiency, but rather what might be expected as the result of long, continuous multiple deficiencies and as secondary manifestations of the extensive liver changes.

This simple experiment demonstrates the possibility that long continued multiple malnourishment may indeed have a closer connection to many diseases that we now generally recognize. This is not to say that malnourishment is the direct and only factor in cirrhosis or any of the other diseases previously mentioned. However, experiments such as this indicate how unwise it would be to dismiss nutrition as a factor in the cause or progress of such diseases, especially as they occur in malnourished groups.

It will be particularly interesting to follow the further studies on this problem which are directed toward identifying those factors, either in the diet or missing from it, which are responsible for the high prevalence of cirrhosis in the Bantu. It seems likely that the extensive knowledge derived from studies of the influence of protein, methionine, cystine, and choline contents of the diet on fatty liver in experimental animals will find practical application in the solution of this problem (*Nutrition Reviews* **1,** *88 (1943)*).

Some years ago McCarrison (*Nutrition Abstr. Rev.* **2,** *1 (1932)*), struck by the con-

trast of the well developed healthy appearance of the people of northern India as compared to the poor physique, low endurance and resistance of those in the southern and eastern sections of the country, carried out similar studies. Rats raised on diets typical of the various sections of the country reflected in a remarkable degree the growth, development, and general health typical of the population of the corresponding area.

Orr and Gilks (*Med. Res. Council, Special Report Ser. no.* **155** (*1931*)) have compared the physical development and health of 2 African tribes originally from the same stock, living side by side, alike in most respects except for dietary habits. The Masai are pastoral people and consume much milk and meat, while the Kikuyus live for the most part on cereals with some tubers and legumes. The latter tribe is definitely inferior to the former in physical development. The Masai men are on the average 3 inches taller and 23 pounds heavier, and the Masai women, 3 inches taller and 27 pounds heavier than their Kikuyu neighbors. Furthermore, there is a similar striking difference in prevalence of chronic respiratory disease, parasite infestation, and general health and mortality records. Added evidence that these differences are due to the level of nourishment has been furnished by the prompt improvement of those of the Kikuyu tribe who were changed to a satisfactory diet.

FOOD HABITS IN AN URBAN POPULATION DURING 1943

The importance of proper food in maintaining health during the present emergency makes studies of dietary patterns a matter of general interest at the present time. With an increase in purchasing power of many people, the increased market demand for food and the rationing of certain foodstuffs, information obtained while these factors are in operation is of particular value. Downes and Baranovsky (*Milbank Memorial Fund Quart.* **22,** *161* (*1944*)) report the findings from two records of foods consumed during one week by 943 white families living in the Eastern Health District of Baltimore. The records were obtained in January and February of 1943, and again in April and May; that is, before and after the rationing of processed foods, meats, and fats. The families studied constituted a representative sample of the population of the district except for some weighting with older families. In 59 per cent of these families one or more members were engaged in defense work; in only 6 per cent were there no employed persons in the family, and most of these were in the oldest age group. The amount of money spent for food was recorded. On the basis of $3.60 per adult per week as the minimum weekly expenditure with which a proper diet can be obtained, over 85 per cent of the families were found to be spending enough money on food to enable them to obtain an adequate diet.

The seven-day record was designed to characterize the dietary pattern of the family, and was based on the amount or frequency of use of twelve specific food items which are important in assuring an adequate intake of nutritional essentials in the usual American diet. It was found that the dietary pattern of the family was related to the age of the housewife and the data are divided accordingly into three groups: (1) for families where the housewife was under 40 years of age, (2) for families where the housewife was between 40 and 59 years old, and (3) those where the housewife was 60 years of age or older. The best diets were recorded for the youngest group and the poorest for the oldest families; this was true for most of the specific food groups except for meats and butter.

Citrus fruits and tomatoes were used six or more times a week in from 58 to 78 per

cent of the families. Lean meat, fish, or poultry were eaten at least once a day by most of the families; from 15 to 25 per cent had meat less than six times per week. The use of eggs averaged less than 2.5 per person per week in from 8 to 20 per cent of the families. From 28 to 41 per cent used less than one quarter of a pound of butter or enriched oleomargarine per person per week. From 41 to 49 per cent reported using dark bread at least once a day, but only from 5 to 10 per cent ate a whole grain cereal every day. A relatively high proportion of all families used an "unsatisfactory" amount of milk, with only 30 to 42 per cent using 3.5 quarts per person per week. The use of green and yellow vegetables was infrequent, with 26 to 30 per cent averaging one serving every day, and 13 to 33 per cent using them less than three times per week. The age differential was particularly marked in regard to milk, and green and yellow vegetable consumption.

The comparison of types of food used by the same family at the two different recordings is used as an index of the tendency of the food habits to be fixed and to indicate the reliability of the dietary pattern for one week as typical of the family's eating habits. This was accomplished by measuring the degree of association in the frequency of use of specific foods at the two recordings by the coefficient of correlation. These were statistically highly significant for each food group and in each age class, but the coefficients were of a relatively low order, most of them falling between +0.23 and +0.50. Thus, although there was a definite general tendency for families to be high or low in the use of specific foods at both times, there was considerable variation in the families' use of certain foods or groups from one period to another. The best association in all age classes was in the use of milk, with coefficient of +0.53 to +0.72. It was also noted that the food habits of the older families tended to be less variable. It was concluded that a food record of a family for any particular period cannot be considered as typical of the food habits of the family, a conclusion in accordance with the results of Huenemann and Turner (*J. Am. Dietet. Assn.* **18,** *562 (1942)*) obtained in a detailed recording of the diets of 25 children at three different periods of ten to fourteen days.

There was no evidence that food rationing had an adverse effect on the dietary patterns, or materially altered the food habits. A distribution of the paired differences in use of specific food groups, and analysis of the differences in mean levels of use at the two periods, showed usually a slight and not significant increase. There was no significant change in the use of meat, meat substitutes, or table fats. There was an increase in the use of eggs in the second period, and a definite decrease in the frequency of use of all vegetables, largely accounted for by a decrease in use of white potatoes. These are probably the result of seasonal changes in supply.

To summarize the food patterns in terms of adequacy, the frequency in use of five food groups (citrus fruits or tomatoes, green or yellow vegetables, eggs, milk, and meat) was classified in three categories according to degree of agreement with the dietary pattern of the Food and Nutrition Board of the National Research Council: as (1) satisfactory if used equal to or slightly below the recommended standard, (2) marginal if moderately below standard, and (3) unsatisfactory if considerably below standard. The composite diet was graded as satisfactory only if all five groups were satisfactory, as marginal if one or more groups were marginal but none unsatisfactory, as unsatisfactory if any of the five groups fell into this category. In the 318 families with housewives under 40 years of age, the diet pattern was classified as satisfactory in 31 per cent, marginal in 43 per cent, and unsatisfactory in 26 per cent. In the 432 families with housewives in the 40 to 59 year age group, the figures were 26 per cent satisfactory, 38 per cent marginal, 36 per cent unsatisfactory. In the 193 families with housewives in the oldest age group, the

dietary pattern was satisfactory in only 13 per cent, marginal in 30 per cent, and was unsatisfactory in 57 per cent. These figures are for the first period of study; a slight improvement in all age classes was noted in the second period, but the general pattern and significance was unchanged.

These results are in general accord with the results of recent dietary studies in California (*Nutrition Reviews* **1,** *239* (*1943*)). Both studies indicate that relatively few individuals or families follow a dietary pattern which is adequate with respect to all food groups considered when compared with the recommendations of the Food and Nutrition Board. The tendency of the younger individuals and families to have better diets was encountered in both studies. The inferences regarding the effect of food rationing on dietary habits are limited by the fact that the study was made in the first few weeks after rationing was introduced.

DIET AND CONGENITAL MALFORMATION

That the diet of the mother may be responsible for congenital malformation of the young has been suspected for a long time. Zilva, Golding, Drummond, and Coward observed in 1921 that when sows were fed a diet deficient in vitamin A, young pigs were born in which some of the limbs were missing completely. Hughes has reported deformities in young pigs produced by a purebred gilt fed a diet low in both vitamins A and G. Hale (*J. Heredity* **24,** *105* (*1933*); *Am. J. Ophth.* **18,** *1087* (*1935*); *Texas State J. Med.* **33,** *228* (*1937*)) also fed diets low in vitamin A, and described pigs born without eyeballs. He was able to demonstrate that the defect was not determined genetically, and he also described other abnormalities including harelip, cleft palate, accessory ears, and misplaced kidneys.

Cows fed poor quality roughages low in vitamin A sometimes give birth to blind calves. In this species the essential defect is a constriction of the optic nerve. The hen is another species in which a deficient diet may cause embryonic abnormality. The defects include micromelia and shortness of the skull, and the factor preventing these disorders is contained in wheat germ, liver, and whey. Lepkovsky, Taylor, Jukes, and Almquist (*Hilgardia* **11,** *559* (*1938*)) noted a degeneration of the Wolffian bodies and reduced size of the embryos in eggs laid by hens on a diet deficient in riboflavin.

In the newborn rat congenital defects of the skeleton have been described by Warkany and Nelson (*Science* **92,** *383* (*1940*); *Anat. Record* **79,** *83* (*1941*)); and Warkany, Nelson, and Schraffenberger (*J. Bone Joint Surg.* **25,** *261* (*1943*); *Am. J. Dis. Child.* **65,** *882* (*1943*)), after feeding the mothers vitamin D + a standard ration widely used in the experimental production of rickets and in the assay of foodstuffs for antirachitic potency. Their diet contained yellow corn meal 76, wheat gluten 20, calcium carbonate 3, and sodium chloride 1. Every tenth day 60 I. U. of vitamin D were given as viosterol. The deformed offspring showed cleft palate, shortening of the tibia, mandible, fibula, radius, and ulna, and fusion of ribs, fingers, and toes. The same characteristic pattern of malformation was noted in three different strains of rats, and could be prevented either by feeding an adequate stock diet or by adding 2 per cent of pig liver to the original defective one. The defective diet apparently caused no great damage to the adult females, for after producing abnormal young they could be mated again, and if the liver supplement was fed, normal offspring resulted.

The search for the active principle lacking in the defective diet was complicated by the fact that there is only a narrow range in the degree of deficiency that results in live abnormal young. In more extreme dietary deficiencies, as might be expected, the embryos

die *in utero*; if the diet is not sufficiently defective, normal young result. In the experiments by Warkany and associates, less than one third of the young were anatomically defective, and the defects were less frequent in first litters than in later pregnancies.

More recently, Warkany and Schraffenberger (*J. Nutrition* **27**, *477 (1944)*) have identified the active factor as riboflavin. Preliminary experiments had indicated that an alcoholic extract of liver prevented the defect, but that no activity could be detected in liver ash, casein, iodine, manganese, dried alfalfa, cod liver oil, or wheat germ oil. When mixtures of the crystalline B-vitamins—thiamin, niacin, pyridoxine, calcium pantothenate, and riboflavin—were added to the defective diet, all of 371 young born were normal. When riboflavin alone was added, all of 319 young born were normal. When various combinations of B-vitamins other than riboflavin were added to the defective diet some of the young invariably were abnormal.

Final proof of the importance of riboflavin consisted in the production of abnormal young by mothers fed a synthetic diet deficient in this vitamin. The greatest yield of abnormal young resulted when the deficient ration was fed to adult female rats for about three weeks prior to breeding. Apparently a shorter period did not permit a sufficient lowering of the body reserves, whereas more prolonged periods of depletion completely prevented live births. Thus it would appear that the old rachitogenic diet had produced just the right degree of riboflavin deficiency to make the effect on the young apparent. Yellow corn, of course, contains some riboflavin, and many years ago Steenbock and associates were able to demonstrate that young rats fed rachitogenic diets grew for many weeks when vitamins D and A were also fed.

The means by which riboflavin affects skeletal growth are as yet uncertain. Warkany and Schraffenberger suggest that riboflavin deficiency interferes with the formation of the membranous skeleton which precedes both the cartilaginous and osseous stages of development. In this connection it is of interest that defects of the soft tissues also appear occasionally, although the skeletal defects described are much the more abundant.

THE NUTRITIONAL REQUIREMENTS OF THE DOG

In the opinion of Michaud and Elvehjem (*Nutrition Abstr. Rev.* **13**, *323 (1944)*), the physiologist, the nutrition worker, and the veterinarian, as well as the dog owner, should know what nutrients are essential for the dog and in what quantities. More and more of the difficulties encountered in the normal development of the animal body are being associated with faulty or improperly balanced diets. The dog has proved to be an extremely valuable laboratory animal, not only to the physiologist but to the student of nutrition as well. This animal played an important role in the early studies on experimental rickets and vitamin D, and was used exclusively by Whipple and co-workers in their important studies on blood regeneration after hemorrhage. The successful prosecution of the search for the antipellagra vitamin was made possible by the employment of the dog as the experimental animal.

The caloric requirements of the dog are similar to those of man. It has been estimated that a dog weighing 13.5 kg. needs 80 calories per kilogram, while a child weighing 10 kg. needs about 100 calories per kilogram daily. Since the demand for calories is more directly related to body surface than to weight, it should be kept in mind that the smaller the dog the greater its relative requirements. Brody, Procter, and Ashworth (*Univ. Mo. Agr. Exp. Sta. Bull. no.* **220**

(*1934*)) estimate that an adult dog weighing 50 kg. needs only 49 calories per kilogram daily, while one weighing 1 kg. should receive 141 calories per kilogram. Growth, gestation, and lactation increase the caloric requirements. Although these data give a fairly good indication of the energy needs, Michaud and Elvehjem point out that, in practice, the individual response is often the best guide. If a normal adult animal loses weight, its caloric intake should be increased and vice versa.

The carbohydrate intake may be varied widely if the remainder of the diet contains all of the other essential factors. Good results have been obtained with percentages of carbohydrate varying from 12.5 to 70. There is great latitude also in the type of carbohydrate which may be employed. Many laboratories have used sucrose successfully. Lactose is entirely satisfactory as evidenced by the fact that Anderson, Elvehjem, and Gonce (*J. Nutrition* **20,** *433* (*1940*)) carried dogs through several generations on mineralized milk diets. Either raw or cooked starch is digested almost quantitatively. Potatoes were long believed to be harmful but Roseboom and Patton (*J. Am. Vet. Med. Assn.* **74,** *768* (*1929*)) have shown that they are well utilized. Michaud and Elvehjem suggest that the objection to starch and starchy foods may have arisen from the association of the feeding of dog biscuits to hysteria or "fright disease." The available evidence indicates that although this condition may be brought about by the presence in certain foods of a toxic factor, starch is definitely not the causative agent.

There is very little information about the optimum fat intake for the dog. Most experimental and commercial rations contain 5 to 11 per cent of total fat but the percentage has been raised as high as 40 and lowered to 0.13 without serious ill effects. When the diet is adequate in all other respects, high fat diets appear to improve the quality and luster of the hair coat. Low fat diets tend to produce a flaky dermatitis and coarse, dry hair. Adequate amounts of protein and choline must be provided, however, because of the danger of fatty cirrhosis of the liver. Care must be taken to include adequate amounts of the fat soluble vitamins and the essential fatty acids.

Although its digestive tract is that of an omnivore, the dog is classed as a carnivore. For this reason, meats and other protein foods in its diet have always received a great deal of attention. In recent years our attention has been drawn to the fact that meats contain dietary essentials other than protein and that when these are supplied the value of protein in the diet is directly related to the amino acids released during digestion. Rose and Rice (*Science* **90,** *186* (*1939*)) have reported that the dog will grow and develop normally when supplied with a mixture of nine amino acids, tryptophane, lysine, histidine, phenylalanine, leucine, isoleucine, threonine, methionine, and valine. For blood regeneration, Madden and co-workers (*J. Exp. Med.* **77,** *277* (*1943*)) find arginine as well to be essential. It appears that the essential amino acids in adequate amounts are supplied by 18 to 22 per cent of casein in the diet, for many workers have shown that this level is sufficient even for growing pups. The amount of any other protein required depends on digestibility and the amino acid composition. Melnick and Cowgill (*J. Nutrition* **13,** *401* (*1937*)) give the following relative figures for the biologic value of proteins for dogs: lactalbumin 100, serum protein 80, casein 73, and gliadin 33. The question of digestibility is important in the case of commercial dog foods, particularly at the present time. Due to the protein shortage, these foods are now limited to 24 per cent of protein, of which not more than 8 per cent may come from animal sources. The animal protein is furnished largely by meat and fish scraps. Theoretically, 24 per cent of mixed protein should be adequate, but Newell and Elvehjem (*unpublished data, quoted by Michaud and Elvehjem*) have found that the addition of casein to commercial dog foods

causes an improved growth response in young pups. Most dog breeders maintain that dogs must be given some animal protein but Koehn (*Alabama Polytech. Inst. Agr. Exp. Sta. Bull. no.* **251** (*1942*)) has obtained good growth in dogs on an all vegetable ration containing yellow maize meal, wheat shorts, and peanut meal as the only sources of protein. In practice and considering all dietary essentials, it is of course much easier to devise an adequate diet if animal products are used. Protein deficiency manifests itself in the dog not only by failure of growth and loss of weight but also by other severe disturbances. For example, Weech and Paige have reported (*Am. J. Path.* **13,** *249* (*1937*)) that a dietary level of 1.5 per cent of protein favors the development of peptic and duodenal ulcers.

Among the fat soluble vitamins, it appears that dogs need A, D, and E. Prolongation of prothrombin time has been produced by impairing normal bile production, but it has not been found necessary to supply preformed vitamin K in the diet of normal dogs, probably because of synthesis of this factor by intestinal bacteria.

Vitamin A deficiency causes ophthalmia, bone changes, deafness, nervous symptoms, and skin lesions. Although the quantitative requirements are not well established, it appears that 20 micrograms of vitamin A per kilogram is a generous intake even for the growing dog. Carotene is comparatively well utilized and 40 to 60 micrograms of this substance may be substituted.

The dog was one of the first experimental animals used in the study of rickets (Mellanby, *Med. Res. Council, Special Report Ser. no.* **61** (*1921*)). There is still considerable disagreement, however, concerning its exact vitamin D requirements. Some observers have reported that even 250 I.U. per kilogram per day are insufficient to prevent rickets in heavy breeds of dogs. The abnormally high Ca:P ratio in their rations however may explain their results. With a normal Ca:P ratio (approximately 1.2 to 1), it appears that 10 to 20 I.U. per kilogram of body weight per day are adequate.

It has been generally assumed that dogs require vitamin E and this assumption now has experimental support. Females on a diet deficient in this vitamin developed normally but produced young that were stillborn or were very weak. The few that survived developed muscle dystrophy, hyperesthesia, and alopecia of the head and limbs (Anderson, Elvehjem, and Gonce, *Proc. Soc. Exp. Biol. Med.* **42,** *750* (*1939*)). A weekly dose of alpha-tocopherol representing approximately 1 mg. per kilogram of body weight per day permitted normal birth and development of the pups.

Water soluble factors known to be required by the dog are thiamin, riboflavin, niacin, pyridoxine, pantothenic acid, and choline. Mention of scurvy as a disease of dogs appears frequently in the French literature and occasionally there is a report of the cure of some pathologic condition with vitamin C. Michaud and Elvehjem point out, however, that hundreds of dogs have been reared on synthetic diets containing no vitamin C. It appears definite, therefore, that this vitamin is not required if the diet is adequate in all other respects.

Voegtlin and Lake in 1919 described anorexia, loss of weight, paralysis, and convulsions in dogs maintained on a "vitamin B" deficient diet (*Am. J. Physiol.* **47,** *558* (*1919*)). We now know that these were symptoms of thiamin deficiency. The effect on appetite is particularly striking.

Ten micrograms of thiamin per kilogram of body weight appear to be sufficient and even generous for maintenance but a level between 10 and 25 micrograms is preferable during growth.

The requirements for riboflavin depend on the age and size of the dog. Adult dogs weighing 10 kg. or more may be maintained on as little as 15 micrograms per kilogram. Smaller dogs appear to need somewhat more per unit of body weight. Growing dogs which have passed the period of most rapid

development require 30 micrograms per kilogram, while for very young dogs 50 micrograms per kilogram are insufficient for maximum growth. Sudden collapse, coma, and death as manifestations of acute riboflavin deficiency were described by Sebrell, Onstott and Hunt (*Pub. Health Reports* **52,** *427 (1937)*). A mild anemia, fatty degeneration of the liver, nervous derangement, skin lesions, and vascularization and opacities of the cornea have been reported as symptoms of chronic riboflavin deficiency.

In the research on the antipellagra vitamin, from the classic work of Goldberger to the isolation of niacin and the demonstration of its therapeutic effectiveness by Elvehjem, Madden, Strong, and Woolley (*J. Biol. Chem.* **123,** *137 (1938)*), the dog was the only reliable experimental animal. The use of other animals by various investigators served only to obscure the picture. Shortly after the isolation of niacin, Sebrell, Onstott, Fraser, and Daft showed that 0.12 mg. per kilogram of body weight will just protect adult dogs from blacktongue when they are maintained on the Goldberger diet (*J. Nutrition* **16,** *355 (1938)*). Since this diet contains an appreciable although inadequate amount of the vitamin, figures obtained with synthetic diets are probably more accurate. Schaefer, McKibbin, and Elvehjem (*J. Biol. Chem.* **144,** *679 (1942)*) have used such diets and state that the requirements of adult dogs are 0.20 to 0.25 mg. and of growing dogs 0.25 to 0.36 mg. per kilogram of body weight.

Pyridoxine deficiency in dogs may manifest itself by a microcytic hypochromic anemia or death may supervene without the appearance of the anemia. Both the age of the animal and the completeness of the deficiency appear to be important. Fouts, Helmer, and Lepkovsky (*J. Nutrition* **19,** *393 (1940)*) reported that 60 micrograms per kilogram of body weight would cure the anemia although other investigators failed to obtain complete restoration of the blood picture until liver or crude concentrates were given. Michaud and Elvehjem state that the level of pyridoxine for optimum growth falls between 10 and 60 micrograms per kilogram with the actual value probably not much above the lower figure.

The symptoms of pantothenic acid deficiency in the dog are coma, convulsions, fatty livers, mottled thymus, and gastrointestinal disorders such as gastritis, enteritis, and intussusception (McKibbin, Black, and Elvehjem, *Am. J. Physiol.* **130,** *365 (1940)*). It has been shown in the same laboratory that dogs grow normally when receiving 100 micrograms of pantothenic acid per kilogram of body weight, but die within a month on 40 micrograms per kilogram.

Only a few reports have appeared concerning the effects of a choline deficiency in dogs. Fouts (*J. Nutrition* **25,** *217 (1943)*) described a deficiency state characterized by loss of weight, moderate anemia, dermal and peptic ulcers, fatty cirrhotic liver, and death in dogs fed a diet containing 15 per cent of casein and 30 per cent of fat and supplemented with thiamin, riboflavin, niacin, pyridoxine, and pantothenic acid. Partial clinical improvement followed the administration of large amounts of choline. The deficiency syndrome was prevented by a similar diet containing 41.4 per cent of casein supplemented with 10 mg. of choline per kilogram of body weight in addition to the other vitamins. Since the body may form choline from methionine and ethanolamine, the dog's need for dietary choline depends on the amount and kind of protein ingested. After consideration of all available data, Michaud and Elvehjem conclude that some dogs can synthesize sufficient amounts of choline to satisfy their requirements but that a level of 10 to 50 mg. per kilogram of body weight daily may be required in most diets.

Of the common minerals, it appears that the dog definitely needs calcium, phosphorus, sodium, chlorine, potassium, magnesium, iron, copper, iodine, and probably manganese, cobalt, and zinc. There is little information concerning quantitative requirements and the little which is available con-

cerns only calcium, phosphorus, iron, and copper. The ratio between calcium and phosphorus seems even more important than the absolute amounts in the diet. A ratio of 1.2:1 of the completely assimilable elements appears to be most favorable for normal bone formation. An excess of calcium increases the demand for vitamin D and finally a ratio is reached where it is almost impossible to prevent rickets. The requirements for iron and copper appear to be 1 to 3 mg. and 0.2 to 0.6 mg. respectively per kilogram of body weight.

Michaud and Elvehjem state, in summary, "One would expect a dog to grow and develop on meat diets if sufficient quantities of internal organs are included to supply extra amounts of vitamin A and riboflavin, and if bones are included to supply additional amounts of calcium. One would expect that iron, copper, manganese, and vitamin D would have to be added to a diet made up largely of milk. Actually, dogs have been carried through several generations when these supplements were added to raw whole milk. If the diet is made up largely of cereals, several additions must be made to render it complete. Sources of protein, riboflavin, nicotinic acid and calcium must be added. The protein may be added as meat or meat scrap, the calcium as bone meal or calcium salts, and yeast or liver may be used for the B vitamins."

The authors conclude that although a few other dietary factors may have to be added to those discussed in this review and the quantitative figures may have to be adjusted somewhat, the picture as presented is essentially correct and complete. They point out that hundreds of dogs have been reared on purified diets of known composition. It should be kept in mind, however, in evaluating these conclusions, that the "purified" ingredients of our so-called "synthetic" diets may be far from pure. It is possible, also, that other factors such as the vagaries of intestinal synthesis may render the completeness of our knowledge more apparent than real.

PREVENTION OF FATTY LIVER

The "normal" liver contains but 4 to 5 per cent fat. In man, a variety of clinical conditions, and in animals a great number of experimental conditions, lead to an increase in the amount of liver fat. Under some circumstances the fat may attain values of 30 per cent or more of the wet weight, in which case well over half of the total liver solids are comprised of fat. Although moderate increases in hepatic fat may not be harmful, the accumulation of large amounts of fat definitely injures the liver and, according to some workers, may lead to cirrhosis. Except for certain rare diseases encountered in man, the extra fat is predominantly triglyceride with some minor although perhaps significant changes up or down in the other lipids.

Within the past twenty years great progress has been made in the experimental production of fatty livers and the elucidation of the factors which will prevent them.

The liver appears both to oxidize fat and to act as a halfway station for fat being transported from the intestinal tract or fat depots to the rest of the body. The various types of fatty livers may be divided into those produced (1) by an increase in the rate at which fat is supplied to the liver or (2) by a decrease in the rate at which the liver is able to dispose of fat. An increased supply of fat appears to be responsible for the fatty livers produced by (a) a high fat diet, (b) starvation (accelerated transport from depots) and (c) stimulation with the "ketogenic" fraction of the anterior lobe of the pituitary (also accelerated transport from depots). Theoretically, at least, an in-

creased liponeogenesis from carbohydrate and protein might also supply fat to the liver faster than it could be handled. A delay in removal of fat from the liver by oxidation or by transport seems to account for the fatty livers produced by (d) poisoning with carbon tetrachloride, yellow phosphorus, etc., (e) a high cholesterol diet, (f) depancreatization + insulin (dogs) and (g) choline deficiency combined with a low protein diet.

The factors which will prevent some of these types of fatty liver—the so-called "lipotropic" factors—have been recently reviewed by McHenry and Patterson (*Physiol. Rev.* **24,** *128* (*1944*)). The authors deal chiefly with the effects of choline, inositol, and lipocaic, with some emphasis on the interesting experimental conditions under which certain of the vitamins are actually conducive to the production of fatty livers. The original article also discusses other effects of choline deficiency besides the production of fatty liver, but these will of necessity be omitted from this summary.

The production of fatty livers by choline deficiency has proved to be very interesting and has provoked thorough study. On a choline deficient diet supplied with all the other nutritive essentials but containing only 10 per cent casein or less, rats develop fatty livers which may be cured either by adding choline to the diet or by increasing the casein percentage. Studies on the factor in casein responsible for the choline-like effect revealed that methionine has a marked lipotropic action which du Vigneaud, Chandler, Cohn, and Brown (*J. Biol. Chem.* **134,** *787* (*1940*)) proved was due to its ability to furnish methyl groups for the synthesis of choline *in vivo*. Thus a deficiency of methyl groups leads to the production of fatty liver. Many other compounds containing labile methyl groups besides choline and methionine are lipotropic. However, it appears that lecithin, which contains choline, is the final active material since arseno-choline, the methyl groups of which are not labile, is nevertheless lipotropic; and, furthermore, when fed, its arsenic can be found in the lecithin of the tissues (Welch, *Proc. Soc. Exp. Biol. Med.* **35,** *107* (*1936*)).

Thus a choline or methyl group deficiency results in a lecithin deficiency and an inability on the part of the liver to dispose of fat at the usual rate. The present evidence suggests that the defect lies in delayed fat transport rather than decreased fat oxidation. This agrees with the original theory of Leathes and Raper ("The Fats," *Longmans, Green, and Co., London* (*1925*)) that phospholipids are necessary for fat transport from the liver.

Although choline deficiency will produce a fatty liver even on a fat free diet, as might be anticipated, a fattier liver is produced on a diet in which in addition the supply of fat to liver is increased; e.g., on a starvation or high fat diet, or if the disposal mechanisms of the liver are further handicapped as by a high cholesterol diet. Conversely, choline may be expected to improve a fatty liver only if the diet is deficient in choline, and can hardly be expected to restore the fatty liver to normal in the face of independent factors tending to flood the liver with fat or handicap fat disposal. Thus, choline is only partially effective in overcoming the starvation or cholesterol fatty liver, or the fatty liver resulting from poisoning with phosphorus.

It is of great interest that inositol, a recently proved component of another mammalian phospholipid, lipositol (Folch and Woolley, *J. Biol. Chem.* **142,** *963* (*1942*)), is lipotropic under special circumstances. It is somewhat active in the case of the cholesterol fatty liver, and perhaps the fatty livers of depancreatized dogs, but it is particularly effective in preventing the fatty liver which appears during the first week following restoration of the known vitamins to rats previously deprived of the water soluble vitamins.

A third lipotropic factor, lipocaic, described by Van Prohaska, Dragstedt, and Harms (*Am. J. Physiol.* **117,** *166, 175* (*1936*)),

is effective in preventing the fatty liver which develops in depancreatized dogs maintained with insulin. Lipocaic is a crude mixture obtained from pancreas, but since its action in this case cannot apparently be accounted for by its content of small amounts of choline and inositol, it must contain an unidentified lipotropic material. The product supplies a significant amount of protein, as generally used. Effects of lipocaic on other types of fatty liver, however, appear to be accounted for by the presence of definite amounts of inositol.

A most interesting type of fatty liver has been produced by McHenry and associates by the following technic: For three weeks rats on a fat-free, 10 per cent casein diet are deprived of choline and all water soluble vitamins, following which the diet is supplemented for one week with various test materials and the liver is then analyzed for fat. With no supplement, the liver fat lies in the normal range (3 to 4 per cent). The only single material which will affect the liver during this fourth week is thiamin, supplementation with which results in a moderately fatty liver (8 per cent). This increase is preventable with choline. If the supplement is made more adequate by adding riboflavin, pantothenic acid, pyridoxine, and niacin in addition to thiamin and choline, a 10 per cent fatty liver results in spite of the choline. If biotin + these other vitamins is given during the fourth week, the liver fat reaches the level of 18 per cent with choline or 25 per cent without. According to McHenry, these very fatty livers are completely prevented by giving inositol in addition to choline.

These results are surprising in view of the fact that neither biotin nor inositol are ordinarily required by the rat. Furthermore, it may be pointed out that without a preliminary period of vitamin deficiency and weight loss, the above choline containing diets would not have yielded fatty livers.

The weight gains in the fourth week of these experiments appear to parallel the final liver fat. Thus, during the last week the above rats gained on the average −10, +5, +16, and +25 g., depending on whether the supplement was nothing, thiamin alone, all vitamins except biotin, or all vitamins including biotin. Choline was apparently without effect on the weight gain. Bearing in mind the fact that with a complete vitamin lack, thiamin deficiency is the earliest deficiency to appear in the rat, and that hence thiamin would be the limiting factor at the end of the third week, one is tempted to explain these vitamin effects somewhat as follows: When after a three week period of extreme vitamin deprivation vitamins are restored to the diet, a rapid growth and a rapid synthesis of new fat occurs which overwhelms the fat disposing mechanisms of the liver. It is well known that with the elimination of a deficiency in a young animal a growth burst occurs which exceeds the normal rate. Since before supplementation thiamin is the limiting factor, no other single vitamin by itself will result in either growth or liponeogenesis. When thiamin alone is added to the diet, other vitamins begin to limit growth and liponeogenesis. When the rest of the vitamins except biotin are present in the supplement, growth is so rapid that the biotin supply (from the intestinal flora) becomes temporarily inadequate, *i.e.*, biotin is the final limiting factor; and when it too is added to the supplement, the diet is complete, and growth and fat formation are maximal. This maximal growth outstrips the rat's ability to synthesize inositol (or obtain it from intestinal flora). The resultant temporary inositol deficiency is evidenced not by a limitation of growth, but by a limitation in the ability of the liver to dispose of the flood of new fat, which ability is restored by supplementation with inositol.

This suggested explanation is undoubtedly oversimplified, although it seems to be in keeping with present concepts of vitamin physiology. It is possible, for example, that the special purified diets used affect the intestinal flora in such a manner as to

decrease biotin and inositol production, thereby making easier the production of their respective deficiencies.

Two groups characteristic of phospholipids, choline and inositol, have been shown under certain circumstances to be lipotropic. It would be interesting to see if components of other phospholipids might become limiting factors under special circumstances. Sphingosine has apparently not been extensively tested in this regard. Serine and ethanolamine are ineffective in alleviating a choline deficiency and are perhaps too readily synthesized by most animals ever to become limiting factors. It is of some interest that cysteine, a proved possible precursor of serine, accelerates the rate of turnover of phosphate in liver phospholipids, although serine itself appears to be without such action.

Studies of the production of fatty livers and their prevention have thus already been highly fruitful. It is not unlikely that further studies of fatty livers may greatly advance our knowledge of the intermediary metabolism of fats, which is curiously so much more rudimentary than that of carbohydrates and proteins.

"PYRUVIC ACID" BLOOD LEVELS IN HEPATIC, THYROID, RENAL, AND NEOPLASTIC DISEASE

In comparison with what is yet to be learned, our information concerning the development of nutritional deficiency in association with various diseases is meager. The lack of information is in large part due to the fact that adequate methods of study have not been available.

The study by Davis and Bauer (*Arch. Surg.* **48,** *185 (1944)*) in which data are offered concerning "vitamin B_1 nutrition" in patients with various diseases, unfortunately is open to the criticism that the method used for determining the "pyruvic acid" content of the blood (Lu, *Biochem. J.* **33,** *249 (1939)*) is not specific. Various metabolic by-products falling under the classification of "bisulfite-binding substances" give the same reaction by this method as that interpreted as demonstrating "pyruvic acid." It is not clear whether the investigators appreciated the nonspecificity of the chemical procedure which they employed. Furthermore, a variety of factors probably unrelated to thiamin deficiency influence the values obtained (Weiss and Wilkins, *Ann. Int. Med.* **11,** *104 (1937)*) and it is not known whether these were given consideration in the studies described by Davis and Bauer.

It is interesting, nevertheless, to note a few of the observations they made. In a control series of 30 patients they noted "pyruvic acid" blood levels of 0.5 to 1.3 mg. per 100 ml. Of 32 persons suffering from various forms of hepatic disease, 27 were found to have blood levels of 1.5 to 4.25 mg. When these results were classified according to those found in patients whose hepatic function was regarded as impaired and those in whom it was regarded as normal, the higher blood levels of "pyruvic acid" were found in the patients who showed "clinical" evidence of diminished liver function. The authors also describe the response to the intramuscular administration of 15 mg. of thiamin hydrochloride. Three hours following the injection of thiamin, the "pyruvic acid" blood level was, on the average, 0.28 mg. lower than before the injection in the control subjects and 0.60 mg. lower in those with hepatic disease and normal liver function. In patients with hepatic disease and diminished liver function the reduction of "pyruvic acid" was only 0.13 mg.

Davis and Bauer in a second paper (*Arch. Surg.* **48,** *190 (1944)*) record measurements of "pyruvic acid" in thyroid disease. Seven patients suffering from nontoxic goiter

showed blood levels of 0.45 to 1.3 mg. On the other hand, 16 patients suffering from toxic diffuse goiter or toxic nodular goiter were cited as having 1.45 to 3.5 mg. "pyruvic acid" in the blood. It is noteworthy, however, that only 4 of these persons had levels of 2.1 mg. or higher. When patients with thyrotoxicosis were given iodine, the average blood level was reduced slightly (0.36 mg.). In another group of patients, when thiamin hydrochloride as well as iodine was given, a more substantial reduction in average "pyruvic acid" blood level was observed (0.72 mg.). These results are interpreted as indicating a disturbance of the carbohydrate metabolism in thyrotoxicosis and suggesting a deficiency of thiamin.

In spite of the fact that renal disease impairs the excretion of metabolites, the "pyruvic acid" in the blood in 11 persons suffering from various forms of renal disease was low, with only two exceptions (*Arch. Surg.* **48**, *193 (1944)*). In both of these cases the carbon dioxide combining power of the blood was low. In 19 persons suffering from various types of malignant neoplastic disease, elevated blood levels were found in a few. Finally in almost half of 38 persons suffering from various types of infection the blood levels were somewhat high.

While these studies are of interest, it is unfortunate that the investigators seem to have failed to appreciate the fact that the accumulation of a variety of bisulfite-binding substances will lead to an elevation of the "pyruvic acid" blood level by the method of examination they used. The great dependence of the blood pyruvic acid on the degree of physical activity makes interpretation of small changes in this constituent hazardous. The only observation suggesting a relationship between the measurements recorded and thiamin deficiency is the fact that the blood levels in cases of thyroid disease were more reduced after the injection of thiamin + iodine than after iodine alone. In the cases of liver disease the lack of a significant change in the blood level of "pyruvic acid" following injection of thiamin might be interpreted as indicating an inability to utilize thiamin; but the same observation can also be interpreted as indicating that there is no relationship whatever between the substances measured in the blood and thiamin. It is also important to note that, where one deals with such variable circumstances as are found in patients—variations in previous diet, differences in the nature of the underlying disturbance in pathologic physiology, and the presence or absence of associated infections—the number of cases studied is small. The authors' use of statistics unfortunately does not make the results any more significant. Studies made by a more specific technic are needed before the broad generalizations regarding the presence of thiamin deficiency which are made by the investigators can be accepted.

ASCORBIC ACID IN CANNED CITRUS JUICES

Citrus fruits and tomatoes, fresh and canned, are considered the most important and reliable sources of ascorbic acid in the American diet. They constitute one of the basic seven groups of foods which have been featured so much by the national educational program during the past two years. The inclusion of at least one serving of citrus fruits or tomatoes in the diet daily is indeed one of the more important rules for good nutrition.

The relatively simple method for the determination of ascorbic acid has made it possible to investigate the problems involved in the production, processing, and distribution of citrus fruits and tomatoes so that these products might reach the consumer with a maximum content of ascorbic acid.

The influence of variety, soil, climate, degree of maturity, method of handling, and processing procedures have been studied and reported.

There have been recent reports dealing with various phases of vitamin C retention in canned citrus fruit juices. Although a large proportion of the orange crop is used as fresh fruit, it is reliably estimated that more than half of the annual grapefruit production of the country is canned. In the 1942 to 1943 season some fifteen million cases of grapefruit juice were packed in Florida alone.

Moore, Wiederhold, Atkins, and MacDowell (*The Canner* **98,** *24 (1944)* made a study of the ascorbic acid losses in the production lines of twelve Florida grapefruit juice canning plants, representing over half of the total Florida production. At each plant consecutive samples were taken at the extractors, after screening, at the holding tanks, at the filler bowl, and finally from the canned product. The samplings were performed several times in each of the plants. The average maximum and minimum retentions of the vitamin in the different canning plants were 101 and 89 per cent. The average retention in all the plants was 97 per cent. It may be significant that the plant showing the greatest processing loss employed copper alloy fittings which contacted the heated juice. Modern canning procedures for grapefruit juice therefore permit little loss of ascorbic acid. The average value for these canned grapefruit juices was 38 mg. per 100 ml.

It is estimated that much of the canned grapefruit juice may not be consumed for as long as one and a half to two years after packing, hence it is of importance to have a complete knowledge of the retention of ascorbic acid in the canned product. Ross (*Food Research* **9,** *27 (1944)*) has made such a study. After twelve months of storage at room temperature the vitamin C content was found to range from 70 to 80 per cent of that in the fresh canned product. The time of half loss (50 per cent loss) was twenty-two months. The effect of temperature on loss during storage is pronounced; 89 per cent of the ascorbic acid was retained at refrigerator temperature and only 17 per cent at 37° C. It is calculated that the retention in juice stored in a cool warehouse (17° C.) should be 85 per cent after one year. Development of an off-flavor, particularly in orange juice, paralleled the loss of ascorbic acid. A definite changed flavor developed in four months in specimens stored at 37° C., while refrigerated samples retained good flavor for a year. It is evident that the largest losses of the vitamin in citrus fruit juices may occur during improper storage of the canned juice. Although there is little guarantee to the consumer that a given purchased juice has been properly stored, the better trained palate might aid in selecting the higher vitamin juice.

A final consideration of interest to the consumer is the stability of the vitamin in the juice after opening the can. The importance of this phase is accentuated by the economy effected by the purchase of larger cans of these juices and the consequent necessity of temporary home storage of the juice. Scoular and Willard (*J. Am. Dietet. Assn.* **20,** *223 (1944)*) have studied this phase of the problem. Canned grapefruit, orange, pineapple, and apple juice were tested. The ascorbic acid contents of these juices as purchased were 29.9 (grapefruit), 34.6 (orange), 9.8 (pineapple), and 1.7 (apple) mg. per 100 ml. After one day of refrigeration the average loss of ascorbic acid from the freshly opened cans was 3.0 per cent for grapefruit, 3.7 per cent for orange, 1.1 per cent for pineapple, and 24.1 per cent for apple juice. In three days of refrigeration the loss from grapefruit juice was only 5.6 per cent. The losses were the same whether the juice was stored in the opened can or in a covered glass jar.

These studies strongly support the belief that properly processed and stored canned citrus fruit juices represent dependable and economical sources of dietary ascorbic acid.

THIAMIN IN FAT SYNTHESIS

The biochemical conversion of carbohydrate to fat is recognized in every diet plan, and has been adequately proved by controlled experiments. This metabolic change has further interest in the variety of pathologic conditions in which abnormal fat accumulations occur. Yet perhaps less is known of the details of the mechanism of this conversion than of most other biochemical processes. One of the isolated beliefs concerning this process has been that thiamin plays an essential role. McHenry and Gavin (*J. Biol. Chem.* **125,** *653 (1938)*; *Ibid.* **128,** *45 (1939)*) have for example noted that administration of thiamin to thiamin-deficient pigeons or rats produced an increase in body fat. McHenry (*J. Physiol.* **89,** *287 (1937)*) also reported that fatty liver was not produced in choline deficiency unless thiamin was present, it presumably being necessary to convert the carbohydrate of the diet to fat. Since pyruvate and its metabolic products derived through thiamin catalysis have enjoyed a prominent position in theories of carbohydrate to fat conversion, these conclusions were readily acceptable.

The conclusions drawn from the experiments have been challenged only occasionally and indirectly, for example by the findings that restriction of caloric intake or of salt intake could also restrict the occurrence of fatty liver in choline deficiency. Also, in animals deficient in pyridoxine and pantothenic acid, it was found that administration of these factors was as efficient in promoting body fat accumulation as was thiamin administration in thiamin deficiency. Hence the specificity of thiamin in fat synthesis is questioned; its effects may be due simply to a resultant higher intake of food or indirectly to establishment of other normal balances required for the carbohydrate to fat conversion. Obviously the technic of paired feeding was required, and the application of isotopic analysis was desirable to distinguish the otherwise inseparable processes of increased breakdown or decreased formation.

Boxer and Stetten (*J. Biol. Chem.* **153,** *607 (1944)*) have combined these technics in measuring the amount of deuterium which occurred in the liver fats of thiamin-deficient rats and paired-fed controls after administration of heavy water (D_2O). Three groups of 5 rats each were employed, group A receiving a complete basal diet ad libitum, group B the same but without thiamin, and group C the same quantity of diet as daily consumed by group B but with thiamin present. The basal diet was 84 per cent glucose, 10 per cent casein, salts, and agar, with vitamin supplementation. After sixteen days on this schedule, each rat received an injection of nearly pure D_2O and the drinking water was replaced by 6 per cent D_2O. Five days thereafter the animals were killed, and their livers were pooled and frozen with carbon dioxide. The lipids were extracted with alcohol and ether, and the neutral fats were hydrolyzed and separated from the nonsaponifiable fraction. Saturated and unsaturated fatty acids were separated by lead precipitation, and measured for deuterium content.

The decreased food intake and loss of weight were apparent early in the rats receiving no thiamin. The loss of weight was paralleled however by rats of group C restricted to the same food intake as the thiamin deficient animals. The total fatty acids of these two groups, although one received adequate thiamin, was essentially the same and only one-eighth as much as in the normal control animals. On the basis of previous experiments which proved that animals on a fat-free diet can incorporate in the fatty acid nearly half of the deuterium present in the body water, the authors have been able to calculate the quantity of newly synthesized fatty acids. They found that the normal control rats had deposited 2.25 g.

of fatty acids, as compared to 0.18 and 0.27 g. respectively in groups B and C. The investigators confirmed previous findings that the saturated fatty acids were richer in deuterium than the unsaturated, and that the deuterium was equally distributed in the two halves of fatty acids (which could be so split by virtue of a single double bond). These characteristics were not changed in the fats of the thiamin deficient animals.

In summary, the data indicate that the loss of fat in thiamin deficient animals results primarily from a diminished appetite and food intake. Thiamin can no longer be claimed to have a specific effect on the amount or character of the fats synthesized from carbohydrate.

"VITAMIN P" AND INCREASED CAPILLARY FRAGILITY

The body protects itself against hemorrhage by two principal mechanisms: coagulation of shed blood and maintenance of the integrity of the capillary walls. Both of these defense mechanisms are influenced by nutrition. Vitamin K is required for the formation of prothrombin and, therefore, of a normal clot. Vitamin C aids somehow in the formation of intercellular cement substance without which capillaries become abnormally fragile. More controversial (*Nutrition Reviews* **1,** *214* (*1943*)) is the status of vitamin P which Szent-Györgyi and Rusznyák (*Nature* **138,** *27* (*1936*)) claimed also reduced the tendency of capillaries to bleed in some persons. It was claimed that vitamin P is a constituent of citrus fruits and of certain natural vegetable juices, notably paprika juice. Shortly thereafter, Armentano and co-workers (*Deutsche med. Wchnschr.* **62,** *1325* (*1936*)) identified the effective substance as the flavone glucoside hesperidin.

Since that time, there have been isolated clinical reports claiming therapeutic value for foods containing vitamin P in the treatment of patients with bleeding manifestations. Isaacs (*J. Am. Med. Assn.* **121,** *1306* (*1943*)), for instance, reported that the oral administration of 3 g. of citrus pectin per day caused the bleeding time of 8 patients with various types of thrombocytopenic purpura to return to normal even though the platelet count was unaffected. In the discussion which followed Isaacs' paper, Werch (*Ibid.*) questioned the results since "pectin is completely destroyed in the alimentary tract." Kugelmass (*J. Am. Med. Assn.* **115,** *519* (*1940*)) reported that oral doses of 150 mg. of flavones caused a rise in capillary resistance of children suffering from purpura. More recently, Scarborough (*Edinburgh Med. J.* **50,** *85* (*1943*)) has summarized his experience with a nutritional approach to the correction of abnormal bleeding and has restated his conviction that vitamin P deficiencies occur in man. Scarborough believes that vitamin P deficiency is characterized by petechial bleeding, pain across the shoulders and in the legs, lassitude and undue fatigue, low capillary resistance, slightly prolonged bleeding time, and low serum calcium. The syndrome, he states, is cured by administration of preparations of vitamin P, but not by ascorbic acid. The situation is obviously confused; claims for vitamin P or pectin are made under widely different circumstances and the fate of the material in the intestinal tract is in doubt.

Any additional information, therefore, should be welcomed in the hope that it will help eventually to clarify our understanding of these relationships. Further support for belief that flavones influence capillary fragility is provided by the observations of Griffith, Couch, and Lindauer (*Proc. Soc. Exp. Biol. Med.* **55,** *228* (*1944*)). These authors gave rutin, a flavone glucoside which is structurally similar to hesperidin,

in doses of 20 mg. two or three times a day to 11 patients with hypertension who showed an increased capillary fragility. Rutin is described as a constituent of a variety of plants, including tobacco, garden rue, forsythia, elder flowers, and violets. The material used in the study was extracted from a high quality, flue-cured tobacco. Measurement of capillary fragility was repeated every three or four weeks. Of the 11 patients followed for a period of from twelve to sixteen months, capillary fragility became normal in 8 within two months. One of the 3 subjects in whom the fragility increased developed a hemiplegia four months after beginning medication. There were no other complications in the remaining 10 patients, the blood pressure showed no significant change, and no toxic manifestations were noted. Two subjects stopped taking the rutin after their capillary fragility had returned to normal; within six weeks it was increased again. Reinstitution of therapy again resulted in greater resistance.

There are several factors which make it difficult to interpret the results of all these studies. In the first place, there are unquestionably several different factors which cause capillary fragility to become abnormal, and as yet there is no good way to differentiate them. Secondly, methods of measuring capillary resistance are not adequate. Wide variations occur from day to day in the capillary resistance of normal subjects as it is measured with existing technics. There is a striking absence of control subjects in almost all the clinical reports. Most of the published data dealing with the effectiveness of vitamin P claim that it favorably influences bleeding manifestations of some patients, but observers whose results are negative are much less likely to publish their experiences. There is enough evidence to keep alive interest in hesperidin and related flavone glucosides as factors which possibly influence capillary function, but not enough as yet to convince the critical observer that the relationship has been established. Neither is there adequate evidence that the crude or purified flavones are essential nutrients for experimental animals.

PATHOLOGIC FINDINGS AFTER ADMINISTRATION OF DICUMAROL

It seems clear that in animals and human beings the administration of the hemorrhagic agent Dicumarol (3,3′-methylenebis (4-hydroxycoumarin)) is associated with a decrease in the prothrombin of the blood which is proportional to the dosage given. The mode of action of Dicumarol is not known, but since prothrombin is thought to be formed mainly if not entirely by the liver, the question arises whether the effect of Dicumarol is produced through damage of the liver.

In the early studies on hemorrhagic sweet clover disease in cattle (a condition now recognized as due to the action of Dicumarol derived from spoiled sweet clover), gross lobular lesions in the liver were reported (Schofield, *J. Am. Vet. Med. Assn.* **64,** *553 (1923)*). In a later study by Roderick (*Ibid.* **74,** *314 (1929)*), areas of focal necrosis were seen in half the cases only while in the remainder no abnormalities were found. When the use of Dicumarol in man was proposed, its effects in various animals were studied. Rose, Harris, and Chen (*Proc. Soc. Exp. Biol. Med.* **50,** *228 (1942)*) described the effects of large single doses of the disodium salt of Dicumarol when given to mice, rats, and guinea pigs and of large daily doses for prolonged periods in dogs, rabbits, mice, and rats. They described central necrosis of the liver in about half the rats examined and occasionally this was found in rabbits, mice, and dogs. In 5 out of 21 dogs that were killed with daily doses of 5 to 50 mg. per kilogram,

central necrosis was found. The changes, however, were only slight in 4 of these 5 animals.

A new study of this subject is reported by McCarter, Bingham, and Meyer (*Am. J. Path.* **20,** *651 (1944)*). Twenty-five dogs were given large doses orally or intravenously. As much as 50 mg. per kilogram were given intravenously and 5 g. orally. Four additional dogs were given doses approaching those used by Rose and coworkers, namely 10 mg. per kilogram daily for eight to fourteen days. These 4 animals died at the ends of these periods of time. Animals given large single doses orally or intravenously died in three or four to twenty-four hours.

In some of these animals evidence of gross hemorrhage was observed at various sites including the lungs, pleura, gut, and the subcutaneous and intramuscular tissues. Microscopic hemorrhages were present in almost all of the animals. These were irregularly distributed and occurred in almost all the tissues. There were also characteristic vascular changes which involved the small arteries, arterioles, capillaries, venules, and small veins in both the systemic and the pulmonic circulation. The vessels were dilated; there was marked swelling or even vacuolization of the endothelium with areas of endothelial proliferation, degeneration of smooth muscle, and swelling or rupture of elastic fibers, or areas of hyaline necrosis of portions of the entire thickness of the vascular wall.

Definite lesions were found in the kidneys. There was swelling of the glomerular endothelium, swelling and hyalinization of the glomerular epithelial basement membrane, some swelling of the epithelium, and occasional small glomerular hemorrhages. Most of the animals exhibited lesions in the splenic corpuscles and lymph nodes which consisted of necrosis of lymphocytes, phagocytosis of lymphocytes, hyperplasia of germinal centers, and in some instances exhaustion and skeletonization of lymphoid tissue. In striking contrast to earlier reports, no consistent lesions of the liver attributable to the administration of Dicumarol were discovered.

Since the excessive doses used in these dogs would never be given deliberately to human beings and since in none of the studies had Dicumarol been given for several months as might be done in patients, 6 dogs were given daily or almost daily doses of 1.5 mg. per kilogram after an initial dose of 5 mg. One of these dogs died with gross hemorrhage about the neck after being bitten by another dog. Three were killed at the end of one month and two after two months of observation. In none of the tissues from these 6 dogs was there any toxic vascular changes such as had been observed in the animals receiving much larger doses, and the renal glomerular changes were lacking. Five of the 6 animals, however, showed varying degrees of changes in the lymphoid tissue resembling those seen in the spleen and lymphoid tissue of the animals given large amounts of Dicumarol. A vacuolar appearance of the liver cells in these 6 animals was interpreted as due to the accumulation of glycogen probably resulting from their better state of nutrition after one or two months on the laboratory diet.

Autopsies on 5 human beings to whom Dicumarol had been administered, and who died of other causes, revealed none of the lesions observed in the dogs described above, nor were any other lesions found which could be charged to Dicumarol. These observations appear to absolve this drug of any drastic damaging effects on the tissues of the body, but they fail to explain the mode of action of the drug since not even structural changes in hepatic tissue were demonstrated.

THE ASSESSMENT OF VITAMIN A NUTRITION

The attempts which have been made to determine the prevalence and types of nutritional deficiencies in a group of individuals indicate that the apparent incidence of subnutrition will depend to a great extent on the methods employed and the criteria used in their interpretation. Studies of large groups of subjects have been undertaken in the hope that statistical analyses of the data obtained would aid in establishing standards and selecting valid and practical methods, as well as yielding factual information regarding the nutrition of the group studied. Such a study has been carried out by Youmans, Patton, and associates (*Am. J. Pub. Health* **32,** *1371 (1942)*; **33,** *58, 955 (1943)*; **34,** *368 (1944)*) in a predominantly rural area of Tennessee where a total of 1200 subjects, one-third being Negroes, were examined by the combination of food consumption records, medical history, a physical examination, and various laboratory tests. Reports dealing with the characteristics of the population studied, and the findings with respect to caloric and protein nutrition have been reviewed previously (*Nutrition Reviews* **1,** *302 (1943)*; **2,** *76 (1944)*. The hoped for correlations between dietary records and evidences of deficiency on clinical examination and by laboratory tests were poor with respect to these two dietary principles.

In the study of the vitamin A nutrition in the same population, dietary intake of carotene and vitamin A, expressed as the vitamin, was calculated from standard reference tables. In the medical history, particular attention was paid to symptoms of xerosis, xerophthalmia, dermatosis, night blindness, and prexerosis. On physical examination the condition of the hair, skin, and eyes was noted especially. The authors emphasize that none of the symptoms and signs encountered is pathognomonic of vitamin A deficiency, and that their interpretation is clearly a matter of clinical judgment, based on experience with the manifestations of deficiencies of this vitamin, the range of normal variation, and other causes of similar signs and symptoms. Special procedures included a test of dark adaptation with the Hecht and Schaler adaptometer on all subjects able to cooperate, the minimum of several readings made after twenty minutes of dark adaptation being taken as the threshold of light perception. No preliminary bleaching period was used. Precautions were taken to prevent errors due to the physical characteristics of the apparatus and subjective errors on the part of the subjects or operators. The concentration of vitamin A and carotene in the serum of the last 450 subjects studied was determined by the method of May.

Approximately one half of all the subjects had a vitamin A intake below the Recommended Dietary Allowance of the Food and Nutrition Board of the National Research Council for their age and sex. The incidence of low intakes was greatest in the age groups between 10 and 20 years, where 60 to 70 per cent fell below, and was least in the white children under 6 years and the Negro children between 4 and 6 years, with about 35 per cent falling below. For adults the incidence was 40 per cent for white males, 51 per cent for white females, and 48 per cent for Negroes of both sexes.

In the clinical examination, 204 subjects complained of symptoms of prexerosis, such as dryness, burning, photophobia, asthenopia, and conjunctivitis. These symptoms were much more frequent in adults than in children, occurring in 17 per cent of white men, 28 per cent of white women, and 14 and 18 per cent of the Negro men and women, respectively. The highest incidence was in the Negro girls, between 13 and 20 years of age, of whom nearly half presented these complaints. Night blindness was encountered as a symptom in 60 subjects, almost

exclusively in adults, with equal frequency in whites and Negroes, and most often in white women. Thirty-five subjects presented the characteristic dermatosis of vitamin A deficiency, both the dry, horny, "goose flesh" type and the acneform type being observed. It was found with equal frequency in the white and Negro subjects, was limited to those past puberty, and was most common in the 16 to 20 year age group. Fifty-seven subjects were found with changes in the scleras and conjunctivas, such as conjunctivitis, dryness, muddy and pigmented sclera, which while not very specific, might be attributed to vitamin A deficiency. These also were seen almost always in adults, occurred about twice as often in males as in females, and about twice as often in Negro males as in white males. These various symptoms and signs showed no significant tendency to occur together in the same individual, with the exception of the symptom of night blindness which occurred in 16.7 per cent of subjects with symptoms of prexerosis but only in 5.5 per cent of the entire population. Nor was a vitamin A intake below the recommended allowance any more frequent in those with symptoms and signs of a deficiency than in those without.

In the interpretation of the tests of dark adaptation, a final threshold of 4.0 micro-micro lamberts was conservatively taken as the upper limit of normal.. Nearly half of the entire group exceeded this value, the abnormality occurring most often in adults, 49 and 55 per cent in the white males and females, respectively, and 53 and 62 per cent in the Negro males and females respectively. No significant positive relationship was found between poor adaptation and the symptoms of night blindness or xerosis, or dermatosis. There was a significantly lower incidence of poor adaptation in subjects with changes in the sclera as compared to those without such changes.

The lower limit of "normal" concentration of vitamin A in the serum was taken as 70 I. U. per 100 ml. in subjects over 1 year of age. Thirty-three per cent of the white and 15 per cent of the Negro subjects on whom this determination was done had low values. Among the white subjects the highest incidence was in the age groups below 12 years, where it ranged from 42 to 90 per cent with abnormally low values. During adolescence many of the girls had low values in sharp contrast with the boys. In adults, the percentage of those falling below was 12 and 26 per cent for white males and females, respectively, and 12 and 17 per cent for Negro males and females, respectively. Important was the finding of a vitamin A intake below the recommended allowance in 68 per cent of the subjects with low blood levels, as compared with 46 per cent of those with normal blood levels—a significant difference. There was no correlation of low blood levels with the occurrence of night blindness, symptoms of xerosis, or dermatosis. Strangely enough, an apparently significant inverse correlation was found between low blood levels, physical changes in the cornea, and high adaptometer values. An inspection of the data presented suggests that this is due to the factor of age, low blood values being less frequent, scleral changes and high visual thresholds being more frequent with increasing age.

In commenting on these findings, the authors subject each criterion which they have used to a critical review. They point out the fallacy of assuming that, because there was a deficient vitamin A intake in a certain per cent of the population according to dietary records, there must be a deficiency state existing in a similar number. This presumes a precision both in the accuracy of the dietary records and in our knowledge of the standards of requirement by which those intakes are judged, which is unjustified. This is even more true of vitamin A than of some of the other dietary essentials because of the greater storage capacity for vitamin A.

The use of the test of dark adaptation as an index of vitamin A nutrition can be

criticized on the basis of both accuracy and specificity. The instrument used permits a very exact measurement of rod threshold for light perception and subjective errors, especially under survey conditions, and at the level of slight impairment may cause more unreliability than instrumental error. There is also the probability that factors other than vitamin A nutrition are concerned with visual adaptation to dark. A definite tendency toward elevation of the rod threshold with increasing age was noted by the Vanderbilt workers. On the basis of their observations after administration of vitamin A under comparable conditions and with a similar group of subjects, Youmans and associates infer that in about 50 per cent of the cases the finding of impaired dark adaptation by the technic used represents vitamin A deficiency.

Somewhat greater reliance is placed on the vitamin A level in the serum. Known pathologic conditions which lower the blood level in the face of an adequate intake are insignificant in most population groups and can be detected in the clinical examination, and under conditions of ordinary intake the level is sufficiently stable that it does not give misleading values due to recent alterations in the intake. Assuming that the blood level reflects the state of the body reserves, there is a question as to whether a depletion of body stores constitutes a nutritional deficiency. Youmans *et al.* believe that it does, particularly from the point of view of preventive medicine.

The fact that the symptoms and signs encountered in this study are not pathognomonic for vitamin A deficiency is emphasized. The greater incidence of symptoms is probably due to the lesser specificity of these manifestations. The dermatosis is considered a sign of considerable reliability and significance when properly identified. It is pointed out that, like other characteristic eruptions, "the dermatosis of vitamin A deficiency has features of location, distribution, pattern, size, color, shape and consistency which distinguish it from other eruptions. The hyperkeratotic papule is one of the individual lesions of the vitamin A deficiency dermatosis, but a hyperkeratotic papule, or several of them, is not *the* dermatosis."

The lack of correlation and agreement between the various evidences of deficiency may be in part attributed to the lack of precision of the various procedures, tests, and signs However, there is reason for the lack of agreement also in the fact that the various manifestations may be independent and differing in quantitative significance, representing variations in the severity and the duration of the deficiency. This study supports the viewpoint that tests of the nutritional state must be evaluated according to the stage in the development of a deficiency which they are capable of detecting. It also strongly suggests that the validity of a test for the detection of a nutritional deficiency cannot be established by statistical correlation of several procedures of varying reliability and specificity, but must be founded on observation under known and controllable experimental conditions.

CHOLINE IN ANIMAL AND HUMAN NUTRITION

Choline plays an important role in animal nutrition. A deficiency leads to fatty livers in most species of animals, to hemorrhagic degeneration of the kidneys in young rats, to perosis or "slipped tendon" in chicks and young turkeys, and to cirrhosis of the liver in rats, rabbits, and probably dogs. Its place in human nutrition has not been definitely established but beneficial effects have been attributed to choline in the treatment of human liver cirrhosis.

Recognition of the importance of choline

in nutrition began after the discovery of insulin. It had previously been known that excessive amounts of fat were deposited in the livers of dogs within a few days following pancreatectomy. Administration of insulin brought about the removal of this fat. Allan, Bowie, MacLeod, and Robinson (*Brit. J. Exp. Path.* **5,** *75* (*1924*)) showed, however, that fatty livers again were developed in depancreatized dogs which were maintained for some months on insulin. Hershey (*Am. J. Physiol.* (*Proceedings*) **93,** *657* (*1930*)) demonstrated that this could be prevented by the inclusion of impure lecithin in the diets of these animals. Best, Hershey, and Huntsman (*Ibid.* **101,** *7* (*1932*)) continued the investigation of fatty livers, using rats in which this condition had been induced by high fat diets. They showed that lecithin would prevent this fatty change and identified choline as the active ingredient of the lecithin. Reports of other studies on the production and prevention of fatty livers have been reviewed previously (*Nutrition Reviews* **1,** *168* (*1943*)).

Discovery of the lipotropic activity of choline was followed by the finding by Griffith and Wade (*J. Biol. Chem.* **131,** *567* (*1939*)) that an acute choline deficiency in weanling rats may manifest itself by hemorrhage and necrosis of the kidneys. Accompanying the renal damage there is fatty degeneration of the liver and there may be hemorrhage into the eyeballs and other organs of the body. The histopathologic findings of Christensen (*Arch. Path.* **34,** *633* (*1942*)) have been discussed in this journal (*Nutrition Reviews* **1,** *156* (*1943*)). An interesting feature of the acute deficiency syndrome is that if an animal survives, it is able in large measure to repair all damage even though no change in the diet is made.

Choline deficiency in the chick or turkey poult leads to perosis or "slipped tendon" (Jukes, *J. Biol. Chem.* **134,** *789* (*1940*)). This condition is not specific for choline deficiency, however. It may be caused by a deficiency of manganese, of biotin, or perhaps of unknown factors as well (*Nutrition Reviews* **2,** *50* (*1944*)).

By feeding diets low in choline and low in protein, a number of investigators have succeeded in producing cirrhosis of the liver in rats (*Nutrition Reviews* **1,** *88* (*1943*)). Blumberg and McCollum (*Science* **93,** *598* (*1941*)) reported that this could be prevented by the inclusion of choline in the diet and this finding was confirmed by Daft, Sebrell, and Lillie (*Proc. Soc Exp. Biol. Med.* **48,** *228* (*1941*)). Blumberg, Mackenzie, and Seligson (*Federation Proceedings* **1,** *187* (*1942*)) extended the observations on the preventive effect of choline on hepatic cirrhosis to the rabbit. Fouts (*J. Nutrition* **25,** *217* (*1943*)) reported that dogs which were developing cirrhosis on a low choline, low protein diet showed partial clinical improvement following the administration of large amounts of choline.

Three reports have appeared on the use of choline in the treatment of human liver cirrhosis. Broun and Muether (*J. Am. Med. Assn.* **118,** *1403* (*1942*)) treated patients for more then two years with choline and diets low in animal fats and cholesterol. Results appeared to be favorable. Yater (*Ibid.* **121,** *720* (*1943*)) treated 15 patients with choline and observed no significant benefit. Russakoff and Blumberg (*Federation Proceedings* **3,** *96* (*1944*)) described definite improvement, as evidenced by clinical and laboratory findings, in 7 of 9 patients given supplements of choline in addition to a high protein, high carbohydrate, low fat, high vitamin diet. Enlarged livers were considerably reduced in size, ascites was greatly diminished or disappeared, serum proteins increased, the hemogram improved, the prothrombin time decreased, and liver function tests became normal. Three patients who showed no improvement when treated for three weeks with the high protein diet alone, showed improvement within the first ten days of choline therapy. The authors suggest that in some cases choline may provide an im-

portant adjuvant to the therapy of cirrhosis of the liver.

Evidence has been accumulating that a good diet may be important in the treatment of human liver cirrhosis. Pioneer work in this field was done by Patek and Post (*J. Clin. Invest.* **20,** *481* (*1941*)) who reported beneficial results from a nutritious diet and supplements rich in the vitamin B-complex. Exactly what constitutes the best diet for the treatment of this disease remains problematic, but on the basis of present evidence it seems desirable that choline or its dietary equivalent should be included as one ingredient. It should be remembered, however, that no case of clinical improvement from choline alone has been reported.

AN INTRADERMAL TEST FOR VITAMIN C SUBNUTRITION

Some years ago there was considerable interest in the development of an intradermal test for vitamin C subnutrition. The tests used consisted in the intradermal injection of a solution of dichlorophenol indophenol, thus producing a blue wheal. This dye is bleached by ascorbic acid and the rate of bleaching was used as an index of the vitamin C tissue saturation.

The technic proposed by Rotter (*Wien. klin. Wchnschr.* **51,** *205* (*1938*)) was used by a number of workers. One group, including Portnoy and Wilkinson (*Brit. Med. J.* **1,** *328* (*1938*)) and a number of investigators in India found the method satisfactory. Others, including Poncher and Stubenrauch (*J. Am. Med. Assn.* **111,** *302* (*1938*)); Goldsmith, Gowe, and Ogaard (*Proc. Soc. Exp. Biol. Med.* **41,** *370* (*1939*)); and Wright and MacLenathen (*J. Lab. Clin. Med.* **24,** *806* (*1939*)) found the intradermal test to be entirely unreliable. The last mentioned investigators reported that variations in the decolorization of the dye occurred in the same patient and that readings at different sites frequently varied considerably.

The subject of an intradermal test for vitamin C subnutrition has been raised again in a preliminary report by Slobody (*Ibid.* **29,** *464* (*1944*)). This worker decided on the basis of studies in rabbits that the wheal raised by the injection of the dye should be 4 mm. in diameter rather than 2 mm. as proposed by Rotter. This, he claims, greatly reduces the variations in the decolorization time. Furthermore, he found that the concentration of the dye should be N/300 instead of N/400, the more concentrated solution making the results more dependable since a proportionately greater length of time is required for decolorization.

In a human subject Slobody found that in 5 intradermal tests done at adjacent sites on the forearm, the blue color disappeared in approximately the same time. Two hundred and eighty-five tests were done in duplicate on the same and opposite forearms of children and adults who were taken at random for the test. The test was carried out in a hospital. The plasma vitamin C was measured at the same time and the approximate amount of vitamin C in the diet of the subjects during the days preceding the test was known.

There were 59 subjects whose plasma ascorbic acid level was under 0.3 mg. In 54 of these the blue color of the wheal did not disappear until after fourteen minutes or more. In the remaining 5 the time ranged between nine and five-tenths to thirteen and two-tenths minutes. In 7 instances the skin test times were above fourteen minutes and yet the plasma level of ascorbic acid was greater than 0.7 mg. In 18 instances the skin test times were above fourteen minutes while the blood level ranged between 0.35 and 0.55 mg. per 100 ml. In all of the other cases in which the skin test time was less

than fourteen minutes, the blood levels varied widely and showed no correlation with the skin test times.

The author concludes that the proposed skin test gives information as to the body saturation with vitamin C. The discrepancy between the plasma ascorbic acid levels and the results of the skin test is explained on the thesis that the blood level reflects only the recent intake of vitamin C. Thus in 6 of the 7 instances in which the skin test time was above fourteen minutes and yet the blood level was 0.7 mg., ascorbic acid had been taken by the subjects for one or two days preceding the test.

This conclusion, however, seems to be contradicted by the finding that in 45 cases in which 200 mg. of ascorbic acid were given intravenously, the skin test time was reduced in all but one while the blood level of ascorbic acid was raised. This observation suggests a close correlation between plasma ascorbic acid level and the skin test.

Ten children were placed on a diet which contained no fruits or fruit juices. The blood vitamin C level fell and the skin test time became prolonged. It "usually" took about nine weeks before the skin test time was over fourteen minutes. The correlation between skin test time and blood ascorbic acid level, as indicated by the one protocol given, was good. It is stated that the gross and biomicroscopic examination of the gums in these children revealed many of the gingival manifestations of avitaminosis C described by Kruse (*Milbank Memorial Fund Quart.* **20,** *290 (1942)*). Two of the children were given ascorbic acid and the skin time was reduced to normal.

The author does not seem to have proved his thesis that the skin test parallels the degree of body saturation. However, even if the test is only a reasonable index of plasma vitamin C level, it may be useful. No comparison is given between the skin test and ascorbic acid saturation tests. Such studies, as well as confirmation of the reported observations by other workers, are desirable.

WARTIME DIETARY PATTERNS

Ferguson and McHenry (*Canad. J. Pub. Health* **35,** *241 (1944)*) draw essentially the same conclusions regarding the effects of rationing in Toronto from comparison of diet records obtained in January, 1942, and in January, 1944. In each period, food consumption records of 75 schoolchildren between the ages of 14 and 18 years were obtained for one week, the nutritive values calculated, and the adequacy of the supply of different nutrients tested by comparison with the daily allowances of the National Research Council. On this basis, the 1944 food supplies of the girls appeared definitely improved with regard to all nutrients except protein, vitamin A, and riboflavin; the boys showed marked improvement in calcium, iron, and ascorbic acid intake, slight improvement in calories, vitamin A, thiamin, and riboflavin, and no change in protein. However, the children were 2 years older at the time of the second study, and the recommended allowances by which the adequacy of the diets was judged had changed for many of the subjects, being definitely lower for about 50 per cent of the girls and definitely higher for 83 per cent of the boys. On the basis of absolute value of the various nutrients, both boys and girls had definitely greater supplies of ascorbic acid in the second study, with an average increase from 46 to 56 mg. a day for girls, and from 52 to 68 mg. for boys. The boys had larger actual intakes of total calories and of protein, in accord with their increased needs, but the girls did not show a decrease in total food

consumption which would be expected from the sharp drop in the recommended allowances. Both sexes showed slight increases in supplies of calcium, thiamin, and riboflavin. The boys not only increased their total food intake but definitely improved the nutritional quality of their diet.

A comparison of the consumption of particular foods in the two periods is of interest. The use of citrus fruits increased 35 per cent for the girls and 76 per cent for the boys. Milk consumption by the boys increased 27 per cent, but remained unchanged for the girls. Both sexes ate more bread in 1944 but the proportion of brown bread eaten remained the same. Meat consumption showed a 10 per cent decrease among the girls and a 14 per cent increase among the boys but for both sexes the protein furnished in the diet exceeded the recommended allowance. The authors conclude that rationing had not caused any deleterious effect upon the food supplies of this group, and suggest that the improvements which were found were the result of educational efforts and of an improvement of economic conditions.

Another study of dietary patterns of schoolchildren, while less accurate because of the methods used in obtaining the records, is of interest because it compares food habits in World War I with those during the present conflict. Evans and Lubschez (*J. Pediatrics* **24,** *518 (1944)*) obtained records of every article of food eaten on one day from 531 grade school children in New York City in January, 1917, and on 277 children in the same school in December, 1942. The age and sex distribution of the two groups were comparable, and the neighborhood had remained characteristic of the while collar class in New York City. A random sample of 100 records in each group is presented in terms of the per cent of children having stated portions of particular foods. The most striking changes consisted in an increase in the consumption of milk, with 69 per cent drinking 2 glasses or more daily in 1942 compared to 24 per cent in 1917, and in citrus fruits, with 65 per cent having at least one serving in 1942 compared with 25 per cent in 1917. The increased milk consumption was accompanied by a decrease in the use of coffee from 50 per cent to 5 per cent, and in tea from 32 per cent to 3 per cent. The most striking difference in vegetable consumption was an increase in the use of green and yellow vegetables from 32 per cent having one or more servings in 1917 to 90 per cent in 1942. No significant difference in the over-all consumption of meat and meat substitutes was noted. Eggs and potatoes were eaten with equal frequency in each period, and the average consumption of bread decreased from 6 slices a day in 1917 to 4 slices a day in 1942. The intake in terms of nutritional constituents calculated on the basis of the average dietary pattern showed an increase in calcium intake from 0.4 g. to 0.7 g. a day, in vitamin A from 5166 I. U. to 8897 I. U., and in ascorbic acid from 50 to 118 mg. per day. There were only small increases in average thiamin and riboflavin intakes and no particular differences in the protein, caloric, or niacin values of the diet. It is pointed out that the diet pattern in 1942 does not yet indicate an optimum daily diet when compared with the recommended allowances.

All of these studies are subject to the limitations of the use of food consumption records in estimating the state of nutrition. Their value is increased by the fact that they at least compare records obtained by the same method of recording on two different occasions, and in the first two communications reviewed, the same subjects were used on each occasion. They furnish some basis for the belief that food rationing has not been harmful and that worth while results in improving dietary habits can be obtained by education in nutrition.

NOTES

Nutricion

Nutricion is the Spanish edition of Nutrition Reviews—an exact translation prepared, printed, and distributed from Mexico City by La Prensa Medica Mexicana. Nutricion is published in the interests of medical and public health education in the Spanish speaking Americas and in the promotion of cultural relations. It is hoped that scientific institutions interested in nutrition will be found to sponsor Nutricion in each of the Spanish speaking countries. Subscriptions to Nutricion are the same as to Nutrition Reviews, namely, $2.00 per year. All subscriptions should be sent to La Prensa Medica Mexicana, Queretaro 97, Mexico, D. F.

Letters To The Editor

Dear Sir:

The article "Standards for Interpretation of Dietary Surveys" in the September issue of Nutrition Reviews, page 264, reflects certain serious weaknesses in much contemporary thought about the level of nutrition and its measurement and relation to health.

In the first place, it is logically indefensible to speak of failure "to obtain consistent correlation between dietary studies and findings on medical examination," since the dietary intake and the clinical condition *are* related as cause and effect. The two methods of study lead to parallel sets of observational data about a subject or group of subjects. If we proceed to deduce opposing conclusions from the two sets of data, it can mean only that the data are not sufficiently precise, or that the underlying logical process is faulty. And the fault which is commonly made is not far to seek. It is not yet possible to deduce the existence of a vitamin deficiency condition from knowledge of a daily intake less than some more or less arbitrarily fixed amount, because we do not have the necessary information describing the clinical condition of human beings ingesting various set quantities of the given vitamin, both above and below the arbitrary standard. For, ultimately, all statements concerning the presence or absence of vitamin deficiencies (or "subclinical vitamin deficiencies") must rest on clinical (including biochemical and physiologic) findings, and not on food intakes unless and until they can be equated with the clinical findings.

Even then it will not be possible to say with certainty that a given individual having a low intake of a vitamin suffers from deficiency of that vitamin; it will be possible only to state that there is a certain fiducial probability that the individual is not within the normal range: and this for two chief reasons: first, on account of the biologic variation inherent in every species; second, on account of the interaction of other dietary constituents and environmental factors with the function of the vitamin under consideration.

It is high time therefore that we cease to diagnose deficiencies on the basis of observation of food intake, and particularly by comparison of observed intakes with the National Research Council Recommended Daily Allowances which (it cannot be too often repeated) were estimated to allow a considerable safety factor over and above the amount of each vitamin necessary to maintain health. Neither are there any other figures which we can at present accept intelligently as representing average daily vitamin requirements; nor will there be, until considerable numbers of human subjects have been maintained for long periods

on diets with various levels of vitamin intakes and their clinical condition followed closely. Even the clinical criteria of early deficiency of the vitamins are not yet generally agreed upon, so that the very foundation of knowledge for such diagnostic procedures remains to be constructed.

One criterion however, which will certainly prove unacceptable, is that anything short of "saturation" with a vitamin represents a state of deficiency. There is no more reason to support this view than to suppose that an individual is fat deficient because he does not carry the maximum possible amount of fat which can be hung upon his frame. Acceptance of such a criterion can only lead to such confusion of ideas as is displayed by the meaningless concept of "maximum requirement" employed in your review.

Sincerely yours,

W. J. Dann,
Associate Professor of Physiology,
Duke University,
Durham, N. C.

More Vitamin Inhibitors

NUTRITION REVIEWS has previously reported on "vitamin inhibitors," substances which produce a vitamin deficiency in bacteria or animals in spite of an apparent adequate supply of the vitamin (*Nutrition Reviews* **2,** *59, 102 (1944)*). These synthetic compounds have chemical structures similar to the vitamin and apparently displace the vitamin from its normal physiologic role.

Woolley (*J. Biol. Chem.* **154,** *31 (1944)*) and Kuhn, Weygand, and Möller (*Ber. deutsche chem. Gesellsch.* **76,** *1044 (1943)*) have now added riboflavin to the list of vitamins for which a vitamin inhibitor has been synthesized. The latter workers produced riboflavin deficiency in bacteria with a riboflavin analogue in which the methyl groups are replaced by chlorine atoms. Woolley has produced a more complex analogue in which the principal change is replacement of the pyrimidine ring of riboflavin by a benzene ring. Such "phenazine" compounds were shown to produce riboflavin deficiency in bacteria and mice. Dittmer, Melville, and du Vigneaud (*Science* **99,** *203 (1944)*) and Dittmer and du Vigneaud (*Ibid.* **100,** *129 (1944)*) have also reported several antibiotin compounds which inhibit growth of certain bacteria and whose action is overcome by biotin.

We now have the following combinations of vitamin and vitamin-inhibitor compounds:

p-aminobenzoic acid—sulfanilamide
thiamin—pyrithiamin
pantothenic acid—thiopanic acid
vitamin K—3,3′-methylenebis (4-hydroxycoumarin)
—salicylic acid
vitamin C—glucoascorbic acid
biotin—desthiobiotin
—biotin sulfone and others
riboflavin—6,7 - dichloro - 9 - ribityliso-alloxazine
—ribityl phenazine derivatives

A vitamin inhibitor compound is expected to be useful or to be an adjunct to other technics in developing the following objectives: (1) circumventing the use of a purified diet in animals, in which there is a danger of omitting factors other than the one intentionally omitted, (2) avoiding an often monotonous and unpalatable diet of long duration to produce and describe vitamin deficiency states in human subjects, (3) offering a systematic approach to the development of vitamin inhibitors as specific chemotherapeutic agents, insecticides, or fungicides, since some may be expected to interfere more with the metabolism of the parasitic invader than of the host, and (4) providing a specific tool to investigate the metabolic function of vitamins *in vitro*.

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